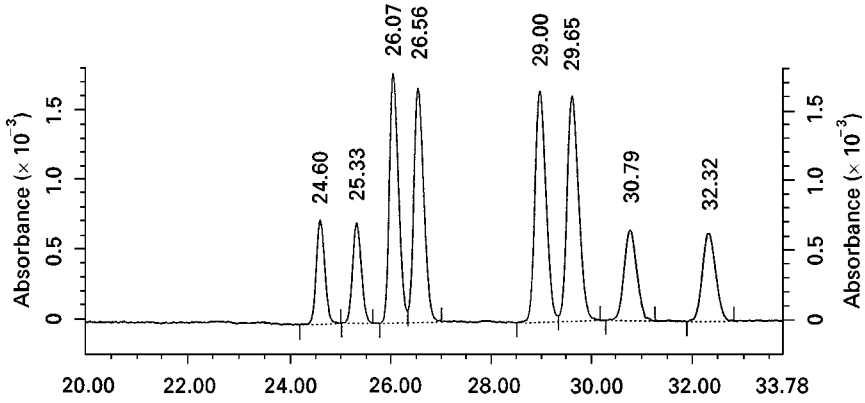


Figure 14 Capillary electrophoresis using a derivatized cyclodextrin as electrolyte additive. Experimental conditions: equipment: P/Ace system 5500 (Beckman); capillary: 50 μm ID uncoated fused silica; total length: 57 cm; length to detector: 50 cm; electrolyte: 20 mM Heptakis (2,3,6-tri-*O*-methyl)*β*-cyclodextrin 10 mM disodium hydrogen phosphate solution adjusted to pH 2.2 with phosphoric acid; analysis: temperature 25°C, voltage + 20 kV, inject sample 2 s, detection UV (220 nm).

The usefulness of cyclodextrins as an electrolyte additive is illustrated in the following example. A substance containing three optical centres, which means eight possible isomers, had to be separated. HPLC experiments on different types of chiral stationary phases did not succeed in a complete resolution of the mixture. The result of a capillary electrophoresis experiment using Heptakis (2,3,6-tri-*O*-methyl) *β*-cyclodextrin as electrolyte additive is illustrated in **Figure 14**.

Compared with HPLC, in capillary electrophoresis many more parameters can be varied to improve separation. Therefore, most of the methods developed on one of the commonly used chiral stationary phases can be replaced by a capillary electrophoresis methods, using cyclodextrins or another chiral auxiliary as electrolyte additive.

See also: **II/Chromatography: Liquid:** Ion Pair Liquid Chromatography; Mechanisms: Chiral; **III/Chiral Separations:** Amino Acids and Derivatives; Capillary Electrophoresis; Cellulose and Cellulose Derived Phases; Chiral Derivatization; Gas Chromatogrpahy; Ion-Pair Chromatography; Ligand Exchange Chromatography; Liquid Chromatography; Molecular Imprints as Stationary Phases; Protein Stationary Phases; Synthetic Multiple Interaction ('Pirkle') Stationary Phases; Thin-Layer (Planar) Chromatography.

Further Reading

Allenmark S (1991) *Chromatographic Enantioseparations, Methods and Applications*. London: Ellis Horwood.

Beesley TE and Scott RPW (1998) *Chiral Chromatography*. Chichester: Wiley.

Bender ML and Komiyama M (1978) *Cyclodextrin Chemistry*. Berlin: Springer.

Chiraldex GC Handbook, 5th edn (1996) Whippany, NJ: Advanced Separation Technologies.

Coventry L (1989) Cyclodextrin inclusion complexation. In Lough WJ (ed.) *Chiral Liquid Chromatography*. Glasgow: Blackie.

Cyclobond Handbook (1996) Whippany, NJ: Advanced Separation Technologies.

Han SM and Armstrong DW (1989) Separation of enantiomers and other isomers with cyclodextrin-bonded phases: rules for chiral recognition. In Krstulović (ed.) *Chiral Separations by HPLC, Applications to Pharmaceutical Compounds*, pp. 208–286. Chichester: Ellis Horwood.

Hinze WL (1981) In: van Oss CJ (ed.) *Separation and Purification Methods*, vol. 10, p. 159.

Kaiser R (1965) *Chromatographie in der Gasphase I*. Mannheim: Bibliographisches Intitut.

DropBooks

Gas Chromatography

V. Schurig, University of Tübingen, Germany

Copyright © 2000 Academic Press

Introduction

The separation of enantiomers (optical isomers) by capillary gas chromatography on a chiral stationary phase (CSP) was discovered by Gil-Av and coworkers at the Weizmann Institute of Science, Israel, in 1966. At the outset of this work, according to Gil-Av,

> this topic was in a 'state of frustration'. Nobody believed it could be done. In fact, people were

convinced that there could not possibly be a large enough difference in the interaction between the D- and L-solute with an asymmetric solvent. This was the feeling people had, even those known as unorthodox thinkers. This view had also some experimental basis, because a number of communications had been published, in which it was claimed that such resolutions could be effected, but nobody was able to reproduce these results, and some of them were shown to be definitely wrong.

Today, almost a reversal of this situation exists. According to the *GC Chirbase data bank*, for most volatile racemic compounds of a variety of different chemical classes, ranging from apolar to polar, an appropriate CSP is available and 22 200 chiral separations by gas chromatography (GC) of 7637 analytes on 684 CSPs (120 are commercialized) have been reported up to the end of 1997.

Three principal CSPs are currently employed; these undergo hydrogen bonding, coordination and inclusion. Modified α-, β- and γ-cylodextrins have proved to be the most versatile and universal CSPs in GC. Anchoring the CSPs to a polysiloxane backbone leads to Chirasil-type stationary phases with improved temperature stability, efficiency and robustness. Immobilization of Chirasil-type stationary phases on the inner column wall furnishes chemically bonded CSPs. Because of the high efficiency, sensitivity and speed, chiral separation by high resolution capillary GC represents a versatile and attractive method for enantiomer analysis. However the prerequisites of the method, i.e. volatility, thermal stability and resolvability of the chiral analytes, restrict its universal use.

The main application of chiral separations by GC is concerned with the precise determination of enantiomeric compositions of chiral research chemicals, intermediates, auxiliaries, metabolites, precursors, drugs, pesticides, fungicides, herbicides, pheromones, flavours and fragrances. As the insight into chirality–activity relationships steadily improves and, as a consequence, legislation on chiral compounds becomes more stringent, the development of reliable methods for the determination of the enantiomeric excess up to 99.9% is of great importance (% enantiomeric excess $= 100(R - S)/(R + S)$, where R is the major enantiomer and S the minor enantiomer). This goal is readily met by enantioselective GC.

Methodology

The separation of enantiomers by GC can be performed in two modes.

1. *Indirect method*. Enantiomers are converted off-column into diastereomeric derivatives by chemical reaction with an enantiomerically pure resolving agent and subsequent gas chromatographic separation of the diastereomers is achieved using a conventional achiral stationary phase.
2. *Direct method*. Gas chromatographic separation of the enantiomers is achieved using a chiral stationary phase (CSP) containing a resolving agent of high (but not necessarily 100%) enantiomeric purity.

While the first method entails the formation of diastereomers before separation, the second involves the rapid and reversible diastereomeric association between the CSP (selector) and the racemic, or non-racemic, analyte (selectand). Since diastereomers display different physical properties, discrimination by incomplete recovery, decomposition and losses may occur during work-up, isolation and sample handling in method (1). Also the detector response can be, in principle, different for diastereomers. Because an achiral detection device does not discriminate between enantiomers, the comparison of relative peak areas employing method (2) provides a direct measure of the enantiomeric composition, provided the detector response is linear over a wide concentration range. Consequently, method (2) is preferred for the determination of enantiomeric excess. This approach requires an efficient selector–selectand system displaying chiral recognition.

Enantioselectivity is governed by the biased diastereomeric association between the enantiomers of the selectand and the chiral selector. Fortunately, by employing capillary columns, efficiency is mostly high enough to resolve racemates having a difference of the Gibbs free energy of diastereomeric association as little as $-\Delta_{R,S}(\Delta G) = 0.025\ \mathrm{kJ\ mol^{-1}}$ (at 25°C), corresponding to a separation factor, α, of 1.01 ($\alpha = k_R/k_S$, with k = the retention factor and the subscript R referring to the second eluted enantiomer and S to the first eluted enantiomer). The measured enantiomeric composition of the analyte determined by method (2) is independent of the enantiomeric purity of the CSP. Lowering the enantiomeric purity of the CSP, however, results in a decrease of α and it is unity when the chiral selector in the stationary phase is racemic. Method (2) is especially useful for the determination of enantiomeric excess when no sample derivatization is required. Owing to the enormous separation power of capillary GC, contaminants and impurities are usually separated from the enantiomers and the simultaneous analysis of the enantiomers of different compounds (e.g. all proteinogenic amino acids) is feasible in one analytical run (cf. **Figure 1**).

Temperature programming and established ancillary techniques such as multidimensional chromatography

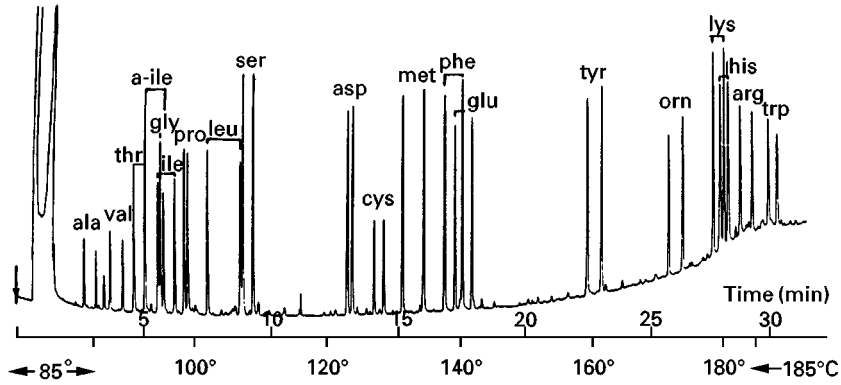


Figure 1 Simulationeous enantiomer separation of 20 proteinogenic amino acids as *N*, *O*, *S*-pentafluoropropanoate-isopropylester (histidine as N^{im}-ethoxycarbonyl) derivatives by GC on Chirasil-Val [**IV**] between 85 and 185°C at 0.35 bar (gauge) hydrogen. Column, 50 m × 0.27 mm (i.d.) glass capillary. D-Enantiomers are eluted before L-enantiomers. (From Bayer E (1983) Chiral recognition of natural products on optically active polysiloxanes. *Zeitschrift für Naturforschung* 38b: 1281–1291.)

and the use of interfacing and coupling methods can readily be adapted to chiral separations. Sensitivity can be extended to the picogram level by electron capture detection or by the combination of gas chromatography with mass spectrometry (GC-MS). GC-MS-selected ion monitoring (SIM) can detect trace amounts of enantiomers in complex matrices.

When the racemate is highly volatile and the chiral separation factor is large ($\alpha > 1.3$), preparative enantiomer separation by GC with packed columns is possible. The development of simulated moving bed (SMB) approaches can be expected in the future. Semipreparative separations can already be carried out at low α values. Recovery from the gaseous carrier is straightforward and pure enantiomers can be obtained even on enantiomerically impure CSPs. For analytical purposes it may be important to differentiate a separation of a chiral compound into enantiomers from the common separation of two achiral analytes. A racemate, when resolved, should produce an exact 1 : 1 peak ratio of the enantiomers. Clearly, only one peak (peak coalescence, $\alpha = 1$) is expected when the racemic CSP is employed. When two CSPs of opposite configurations are applied in different columns, peak inversion (peak switching) can be observed for a chiral analyte in which one enantiomer is in excess.

Classification of Chiral Stationary Phases

Enantiomer separation by GC is mainly performed on three types of CSPs:

- chiral amino acid derivatives via hydrogen bonding;
- chiral metal chelates via coordination (complexation GC);
- cyclodextrin derivatives via inclusion.

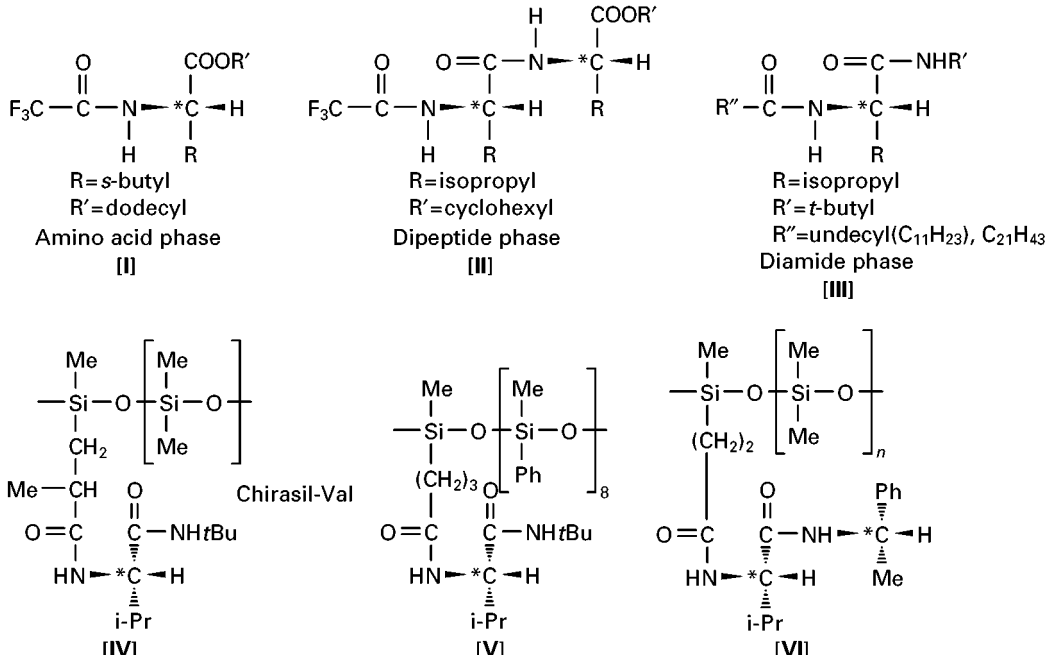
Initially, the chiral selectors were used as involatile neat liquids or as solutions in squalane or polysiloxanes, respectively. Subsequently, a number of chiral selectors have been chemically linked to polysiloxanes (Chirasil-type stationary phases).

Chiral Stationary Phases Based on Hydrogen Bonding

The first successful separation of racemic *N*-trifluoroacetyl amino acid alkyl esters on glass capillary columns coated with involatile *N*-trifluoroacetyl-L-isoleucine lauryl ester [**I**] (cf. **Scheme 1**) was achieved by Gil-Av and co-workers in 1966 and a semipreparative version of this method was reported later. Since then the great potential of this fundamental approach has stimulated continuing research on enantiomer separation not only by GC, but also by other chromatographic techniques such as HPLC.

It was recognized that in the dipeptide phase [**II**] (cf. Scheme 1) the *C*-terminal amino acid was not essential to chiral recognition while the additional amide function was important for additional hydrogen bonding. The second chiral centre was therefore sacrificed by preparing the diamide [**III**], e.g. derived from valine. This chiral selector was subsequently coupled via the amino function to a statistical copolymer of dimethylsiloxane and (2-carboxypropyl)methylsiloxane. The resulting polymeric CSP, Chirasil-Val [**IV**], combining enantioselectivity and efficiency of silicones, exhibits excellent GC properties for the enantiomer separation of chiral compounds undergoing hydrogen bonding. Chirasil-Val [**IV**] is commercially available in both enantiomeric

DropBooks



Scheme 1 Hydrogen-bonding-type chiral stationary phases.

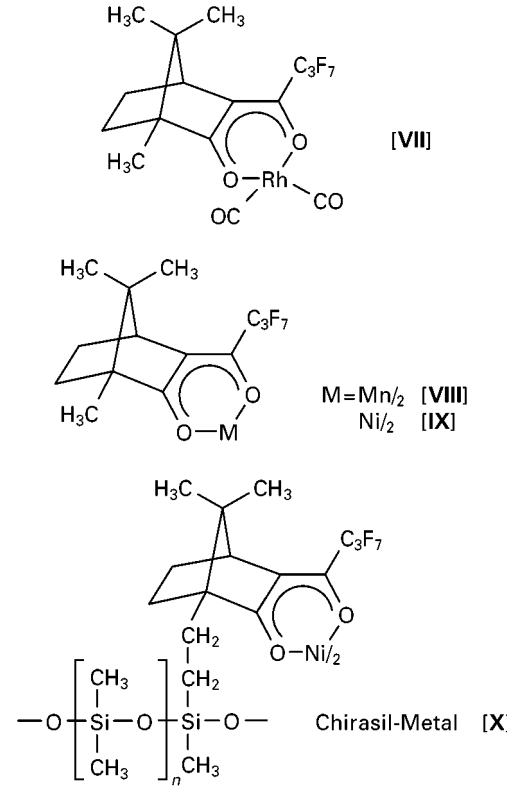
forms. The temperature-programmed simultaneous enantiomer separation of all proteinogenic amino acids in less than 25 min is illustrated in Figure 1. A straightforward approach to polymeric CSPs is based on the modification of cyanoalkyl-substituted polysiloxanes (XE-60, OV-225). For instance the diamide [**III**] was chemically linked to the polysiloxane to give (L)-[**V**]. The diastereomeric selectors (L, *R*, and L, *S*)-[**VI**] contain two chiral centres that enhance enantioselectivity (matched case) or compensate enantioselectivity (mismatched case).

Enantiomer separation by hydrogen-bonding CSPs generally requires derivatization of the analyte in order to increase volatility and/or to introduce suitable functions for additional hydrogen-bonding association.

Chiral Stationary Phases Based on Coordination

The chiral metal coordination compound dicarbonylrhodium(I)-3-trifluoroacetyl-(1*R*)-camphorate [**VII**] (cf. **Scheme 2**) was used for the enantiomeric separation of the chiral olefin 3-methylcyclopentene by complexation GC in 1977. The scope of enantiomer separation by complexation GC was later extended to oxygen-, nitrogen- and sulfur-functionalized compounds using chiral ketoenolate-*bis*-chelates of, among others, manganese(II) and nickel(II) ions derived from terpene ketones such as camphor [**VIII, IX**], menthone, carvone, pulegone, and others after perfluoroacylation.

Figure 2 illustrates the enantiomer separation by complexation GC of simple aliphatic oxiranes, belonging to the smallest chiral molecules. A limiting factor of coordination-type CSPs [**VIII, IX**] is the low temperature range of operation (25–120°C). The thermal stability has been increased by the



Scheme 2 Coordination-type chiral stationary phases.

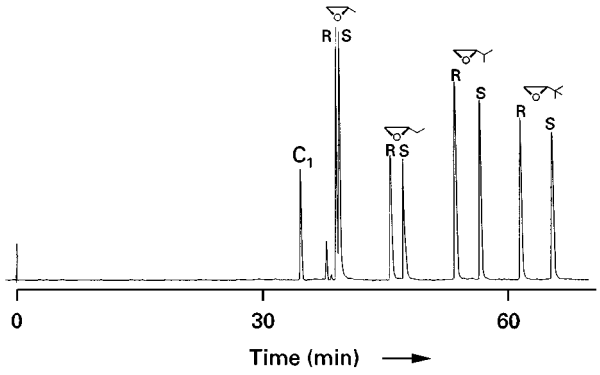


Figure 2 Enantiomer separation of monoalkyl-substituted oxiranes by complexation GC on manganese (**II**) *bis*[3-(heptafluorobutanoyl)-(1*R*)-camphorate] [**IX**] (0.05 molal in squalane) at 60°C. C_1 = Methane. Column, 160 m × 0.4 mm (i.d.) stainless steel capillary. (From Schurig V and Weber R (1981) *Journal of Chromatography* 217: 51–70.)

preparation of immobilized polymeric CSPs (Chirasil-Metal; [**X**] in Scheme 2). In **Figure 3** the GC enantiomer separation of homofuran at 95°C on Chirasil-Nickel [**X**] is shown.

Chiral Stationary Phases Based on Inclusion

The first enantiomer separation using an inclusion-type CSP in GC was reported in 1983 for α- and β-pinene and *cis*- and *trans*-pinane on packed columns containing native α-cyclodextrin in formamide. Later it was recognized that alkylated cyclodextrins (CDs) can be employed in high resolution capillary columns for enantiomer analysis. Thus, neat permethylated β-cyclodextrin, [**XI**] (cf. **Scheme 3**), was used above its melting point and in a supercooled state. Per-*n*-pentylated and 3-acyl-2,6-*n*-pentylated CDs are liquids at room temperature. The CD derivatives, [**XIII**]–[**XVII**], have been used in the undiluted form for the separation of enantiomers of many classes of compounds on deactivated Pyrex glass capillary columns. The more polar CD derivatives, [**XX**]–[**XXIII**], have been coated on fused silica capillary columns.

To combine the enantioselectivity of CDs with the excellent coating properties and efficiency of polysiloxanes, alkylated CDs have been preferentially dissolved in moderately polar polysiloxanes (silicones) such as OV-1701. Thus, the CD derivatives can be employed for GC enantiomer separation irrespective of their melting point and phase transitions. The simultaneous separation of a test mixture of enantiomers of different classes of compounds is depicted in **Figure 4**.

The presence of three hydroxyl groups that can be regioselectively alkylated and acylated offers an enormous number of possible α-, β- and γ-cyclodextrin derivatives, which are not always readily accessible and may require tedious purification steps. Occasionally, CD derivatives such as octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin [**XVII**] are highly enantioselective for the GC enantiomer

DropBooks

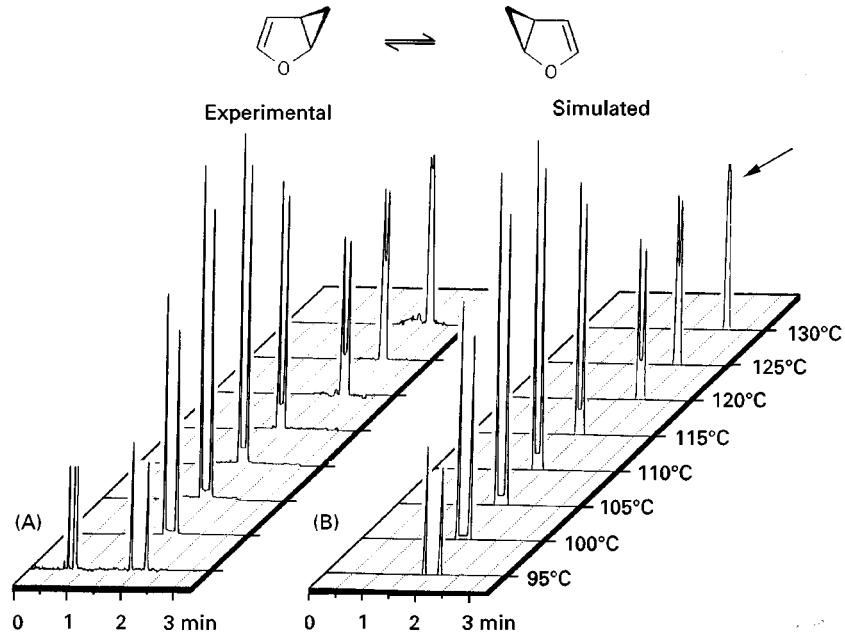


Figure 3 Enantiomer separation of homofuran at 95°C and transient elution profiles at higer temperatures on Chirasil-Nickel [**X**]. (A) Experimental gas chromatograms (column 10 m × 0.1 mm (i.d.) fused silica capillary; film thickness, 0.25 μm). (B) Simulated chromatograms. (From Schurig V, Jung M, Schleimer M and Klärner F-G (1992) *Chem. Ber.* 125: 1301–1303.)

Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin [**XI**]
Heptakis(2,6-di-*O*-methyl-3-*O*-trifluoroacetyl)-β-cyclodextrin [**XII**]
Hexakis(2,3,6-tri-*O*-*n*-pentyl)-α-cyclodextrin (Lipodex A) [**XIII**]
Hexakis(3-*O*-acetyl-2,6-di-*O*-*n*-pentyl)-α-cyclodextrin (Lipodex B) [**XIV**]
Heptakis(2,3,6-tri-*O*-*n*-pentyl)-β-cyclodextrin (Lipodex C) [**XV**]
Heptakis(3-*O*-acetyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin (Lipodex D) [**XVI**]
Octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (Lipodex E) [**XVII**]
Hepatakis(2,3-di-*O*-acetyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin [**XVIII**]
Hepatakis(6-*O*-*t*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin [**XIX**]
Heptakis(*O*-(*S*-2-hydroxypropyl)-per-*O*-methyl)-β-cyclodextrin (PMHP-β-CD, mixture) [**XX**]
Hexakis(2,6-di-*O*-*n*-pentyl)-α-cyclodextrin (Dipentyl-α-CD) [**XXI**]
Heptakis(2,6-di-*O*-*n*-pentyl)-β-cyclodextrin (Dipentyl-β-CD) [**XXII**]
Heptakis(3-*O*-trifluoroacetyl-2,6-di-*O*-*n*-pentyl)-β-cyclodextrin (DPTFA-β-CD) [**XXIII**]

Scheme 3 Cyclodextrin-type chiral stationary phases.

separation of certain racemates (cf. **Figure 5**). Also derivatives containing the bulky butyldimethylsilyl substituent at the lower rim of the CD ([**XVIII**] and [**XIX**]) represent useful complementary CSPs.

A superior class of CSP has been obtained by chemically linking the CD derivatives to the polysiloxane backbone furnishing Chirasil-Dex [**XXIV**] (cf. **Scheme 4**).

Fused silica columns coated with Chirasil-Dex have advantages such as:

- use of a nonpolar polysiloxane matrix (in which CD derivatives cannot be physically diluted) resulting in low elution temperatures for polar analytes;
- high degree of inertness allowing analysis of polar compounds without prior derivatization;
- higher CD concentration resulting in increased separation factors;
- long-term stability with absence of droplet formation leading to loss of efficiency;
- immobilization by crosslinking and/or surface bonding;
- compatibility with all injection techniques.

The rationalization of chiral recognition involving CD derivatives is difficult since almost all classes of chiral compounds, ranging from apolar to highly polar, are susceptible to enantiomer separation on a certain CD-derived CSP, often with no logical dependence on molecular shape, size and functionalities of the selectand and the selector (α, β, γ). Clearly, multimodal recognition processes are important which

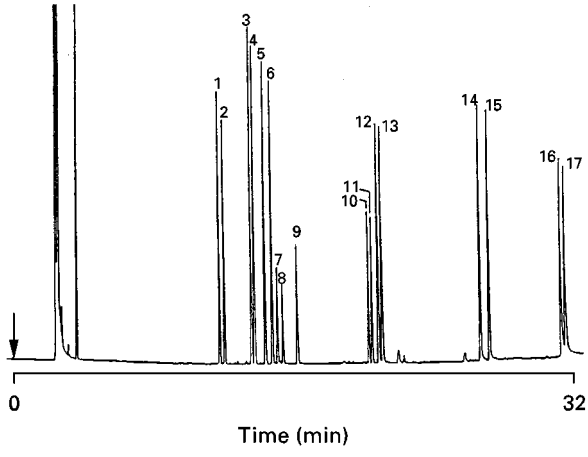


Figure 4 Enantiomer separation of the test mixture α-pinene (1, 2), *trans*-pinane (3, 4), *cis*-pinane (5, 6), 2,3-butanediol (rac) (7, 8), 2,3-butanediol (*meso*) (9), γ-valerolactone (10, 11), 1-phenylethylamine (12, 13), 1-phenylethanol (14, 15) and 2-ethylhexanoic acid (16, 17) by GC on heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin [**XI**] (10% (w/w) in OV-1701) at 50°C and 0.7 bar (gauge) helium. Column, 50 m × 0.25 mm (i.d.) fused silica capillary; film thickness, 0.25 μm. (Courtesy Chrompack International, Middelburg, The Netherlands.)

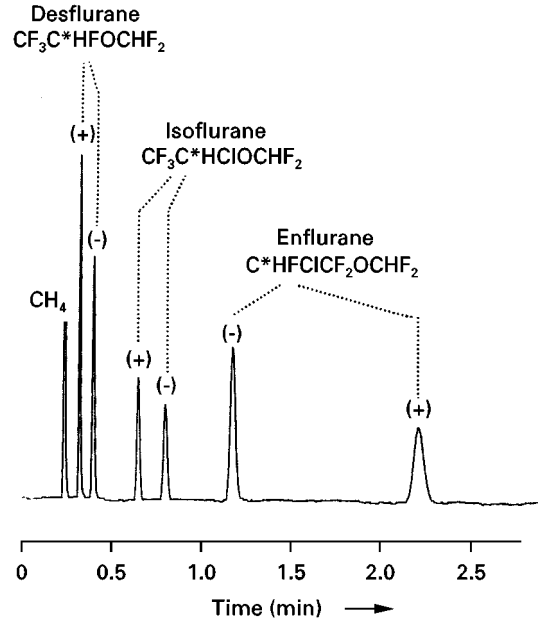
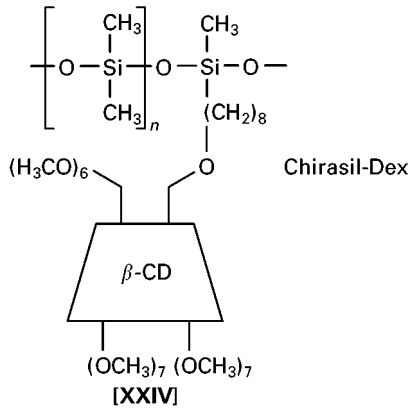


Figure 5 Enantiomer separation of the inhalation anaesthetics desflurane, isoflurane and enflurane by GC on immobilized polysiloxane-bonded octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin [**XVIII**] at 28°C. Column, 10 m × 0.25 mm (i.d.) fused silica capillary; film thickness, 0.18 μm. (From Grosenick H and Schurig V (1997) *Journal of Chromatography A* 761: 181–193.)



[XXIV]

Scheme 4 Chirasil-Dex-type chiral stationary phase.

may involve inclusion, hydrogen bonding, dipole–dipole interactions and dispersion forces. Since enantiomer separations have also been observed with per-*n*-pentylated amylose, inclusion may not be a prerequisite for chiral recognition using CDs. Mechanistic investigations, some of which include molecular modelling studies, have been carried out although no clear-cut rationale for chiral recognition has emerged thus far.

Thermodynamics of Enantiomer Separation

Enantiomer separation by GC is brought about by the difference in the Gibbs free energy $-\Delta_{R,S}(\Delta G)$ of the diastereomeric association equilibria between the enantiomers (selectand) and the CSP (selector). An important prerequisite is a fast and reversible association equilibrium (fast kinetics). The chemical association equilibria in the stationary phase are described by K_R and K_S, with R referring to the second eluted enantiomer and S to the first eluted enantiomer. For enantiomer separation, the Gibbs–Helmholtz equation [1] applies, where R is the universal gas constant, T is the temperature (K), H is the enthalpy and S is the entropy.

$$-\Delta_{R,S}(\Delta G) = RT \ln \frac{K_R}{K_S} = -\Delta_{R,S}(\Delta H) + T\,\Delta_{R,S}(\Delta S) \quad [1]$$

For a 1:1 molecular association, the quantities $\Delta_{R,S}(\Delta S)$ and $\Delta_{R,S}(\Delta H)$ display an opposing effect on $-\Delta_{R,S}(\Delta G)$. At the isoenantioselective temperature T_{iso}, given by eqn [2], peak coalescence (second kind) occurs ($\Delta_{R,S}(\Delta G) = 0$, $K_R = K_S$; no enantiomer separation).

$$T_{iso} = \frac{\Delta_{R,S}(\Delta H)}{\Delta_{R,S}(\Delta S)} \quad [2]$$

Above T_{iso} the sign of enantioselectivity changes, leading to peak inversion (second kind). Below the coalescence temperature, the sign of enantioselectivity $\Delta_{R,S}(\Delta G)$ is governed by $-\Delta_{R,S}(\Delta H)$ and above it, by $\Delta_{R,S}(\Delta S)$. A rare example of the temperature-dependent reversal of enantioselectivity in GC enantiomer separation on a single CSP is demonstrated in **Figure 6**. Usually, even at high temperatures, enantioselectivity is dominated by enthalpy control and separation factors increase with decreasing temperature. Therefore, it is recommended that the lowest possible temperature is used for enantiomer separation by GC.

For undiluted CSPs the quantity $-\Delta_{R,S}(\Delta G)$ can easily be obtained from the separation factor α_{undil},

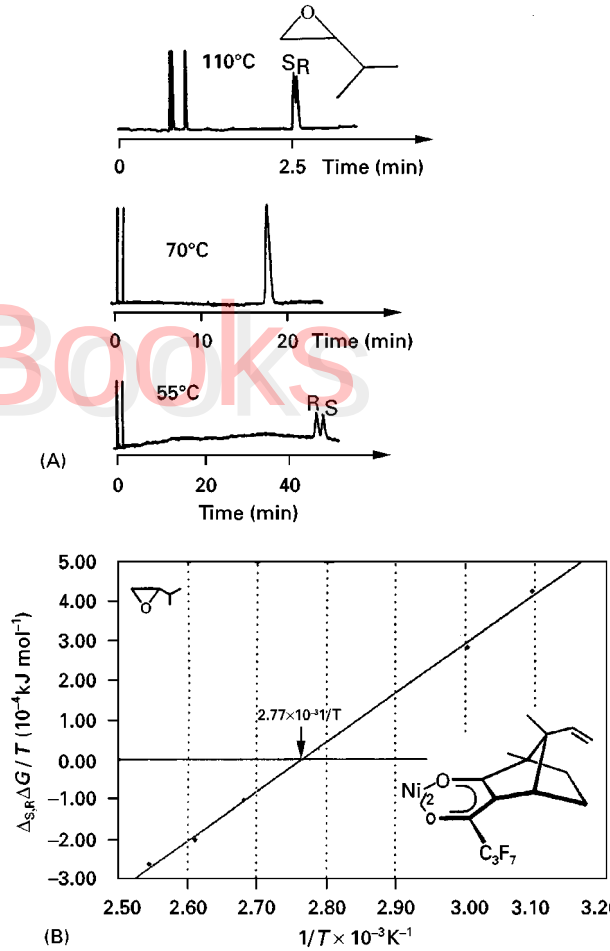


Figure 6 (A) Temperature-dependent peak inversion (second kind) at 55 and 110°C and peak coalescence (second kind) at 70°C during GC enantiomer separation of isopropyloxirane on nickel (**II**) *bis*[3-(heptafluorobutanoyl)-(1*R*)-8-methylene-camphorate] (a derivative of [**IX**]) (0.126 molal in OV-101). Column, 22 m × 0.25 mm (i.d.) glass capillary. (B) Linear van't Hoff plot and determination of T_{iso}. (From Schurig V (1997) In: Jinno K (ed.) *Chromatographic Separations Based on Molecular Recognition*, ch. 7, pp. 371–418. New York: Wiley-VCH.)

DropBooks

according to eqn [3].

$$-\Delta_{R,S}(\Delta G) = RT \ln \alpha_{undil} \quad [3]$$

Although it is occasionally used, eqn [3] is not valid for diluted CSPs because α_{dil} is concentration dependent.

Enantiomerization

The configurational integrity of the enantiomers during the GC process of separation is essential for a correct enantiomer analysis. When enantiomers invert the configuration (or conformation) during separation, transient elution profiles are obtained that are characterized by plateau formation between the terminal peaks of the enantiomers. The barrier of enantiomerization ($\Delta G^{\ddagger}$) can be determined by dynamic GC via peak form analysis of interconversion profiles and the comparison of experimental and simulated chromatograms (cf. **Figure 2**). Only minute amounts of the easily available racemic compound are required. If enantiomerization is fast within the chromatographic timescale, peak coalescence (third kind) occurs (cf. **Figure 2** at 130°C).

Another on-column method for determining interconversion kinetics is based on the 'stopped-flow' technique. In the first part of the column enantiomers are quantitatively separated. Afterwards, the flow is stopped and the column is heated, whereby enantiomerization in the separated fractions commences. After cooling, the flow is restored and the enantiomerized fractions are separated in the second part of the column. From the reaction time and enantiomeric compositions the rate constant can be calculated. Using a combination of three columns, i.e. a separation column, an empty reactor column and another separation column, connected via switching valves for peak-cutting, enantiomerization can be carried out in the gas phase and in the absence of the CSP in the reactor column (enantioselective multidimensional stopped-flow GC).

Assignment of Absolute Configurations by Enantioselective GC

The determination of absolute configurations of chiral analytes is an important task in enantiomer analysis. Absolute configurations of minute amounts of chiral samples may be determined directly, and free of chiroptical evidence, by GC via co-injection of reference compounds with known stereochemistry. Absolute configurations may also be predicted indirectly by empirical rules that correlate the absolute configuration and the order of elution for enantiomers belonging to homologous series of compounds. Although consistent relationships between the order of elution and absolute configuration of congeners have been observed in many instances, remarkable inconsistencies are also known. As a rule, such comparisons, if any, should be restricted to measurements at the same temperature since peak inversion (second kind) may occur at different temperatures as the result of enthalpy versus entropy compensation or multimodal chiral recognition mechanisms. Therefore, the assignment of absolute configurations by GC can be ambiguous.

Method of Enantiomer Labelling

Enantiomers can be quantified in complex matrices when a known amount of the pure enantiomer is added as an internal standard. The pure enantiomer is an ideal internal standard as the enantiomeric excess is not influenced by sample manipulations in diluted systems (achiral derivatization, dilution, injection, detection, chemical and physical losses). The method of enantiomer labelling presupposes the precise knowledge of the enantiomeric excess of the sample and the standards.

Simultaneous enantiomer and isotopic labelling in enantiomer analysis can also be carried in the GC-MS-SIM mode.

Precision and Sources of Error

The precision of enantiomeric excess determined by GC is high over the whole range from 0 (racemic) to 99.9% (nearly enantiomerically pure). At a high enantiomeric excess, the minor enantiomer should preferentially elute as the first peak in order to facilitate correct integration.

Despite the great success of GC for determining enantiomeric excess, potential sources of error should be considered:

- decomposition of the sample during chromatography (the enantiomer which spends a longer time in the column will be lost preferentially, causing an error in enantiomeric excess);
- coelution of impurities accidentally increasing peak areas;
- enantiomerization causing peak distortions (plateau formation);
- peak distortions caused by inadequate instrumentation;
- nonlinear detector response.

In general, the error in enantiomeric excess due to decomposition of the analyte can be reduced if the

difference of the residence time in the column is minimized for both enantiomers. This goal may be realized by using short columns, high pressure drops, elevated temperatures and CSPs exhibiting only small separation factors α. A rather frequent cause for the deviation from the expected 1 : 1 ratio for the racemic mixture consists of the coelution of impurities. This interference can be recognized by determining the enantiomeric excess on two columns coated with CSPs of opposite chirality. The verification of the ideal 1 : 1 ratio of a racemic mixture is always recommended in enantiomer analysis by chromatography. It may also be used to test integration devices.

Practical Considerations

The merit of GC enantiomer separation is the great range of resolvable classes of compounds. With a few exceptions, enantiomer separation by GC is characterized by low separation factors α, and, as a (beneficial) consequence, reduced separation times. The use of highly efficient capillary columns is recommended. Chirasil-Val [**IV**], Chirasil-Dex [**XXIV**], and most cyclodextrin derivatives, [**XI**]–[**XXIII**], coated onto fused silica capillary columns, are commercially available. Factors such as availability, price, performance and reproducibility should guide the analyst when selecting chiral stationary phases for GC. Immobilized chiral stationary phases, such as Chirasil-Dex [**XXIV**], have the advantage of solvent compatibility, resistance to temperature shock and longevity. Enantiomer separation on Chirasil-type stationary phases can be performed in the usual temperature range 25–220°C. For special applications it is also possible to use cryogenic temperatures down to − 20°C.

The dimensions of commercial columns are typically 10–25 m × 0.25 mm (i.d.) and the film thickness of the chiral stationary phase is 0.25 μm. Column miniaturization has important merits. Since enantiomer separation represents a binary separation system, the whole elution window required for multicomponent mixtures need not be exploited unless enantiomers are detected in complex matrices. With shorter columns, the elution temperature can be decreased, so that the chiral separation factor α is increased in the common enthalpy-controlled region of enantioselectivity. The loss of efficiency is compensated by the gain of selectivity leading to comparable resolution factors R_s. The shorter analysis times increase the sharpness of peaks and hence the detectability of the enantiomers. Recommended are 2 m × 0.25 mm (i.d.) columns with a film thickness of 0.25 μm. Further miniaturization via reduction of the internal diameter of the columns to 0.1 and 0.05 mm (i.d.) requires thinner films of the stationary phase in order to keep the phase ratio constant. The reduced amount of stationary phase decreases the sample capacity. The reduced signal-to-noise ratio may become critical in regard to the precision of the enantiomeric excess determination.

Unfortunately, there is no universal CSP available and column selection is a matter of trial and error. Chirasil-Val [**IV**], Chirasil-Dex [**XXIV**] and permethylated β-cyclodextrin dissolved in polysiloxane [**XI**] represent the most popular CSPs. On a given enantioselective column, the parameters column length, temperature, film thickness, concentration of CSP, mobile phase velocity and their influences on the resolution factor R_s (which is governed by the retention factor k, separation factor α and efficiency N) have to be carefully balanced. Some variables cannot be freely selected when commercial columns are used.

While Chirasil-Val [**IV**] and Chirasil-Metal [**X**] are available in both enantiomeric forms, Chirasil-Dex [**XXIV**] and other modified cyclodextrins, [**XI**]–[**XXIII**], occur only in the D form. The strategy of peak inversion (first kind), employed to elute the minor enantiomer as the first peak, is precluded with carbohydrate-based CSPs.

DropBooks

See also: **II/Chromatography: Gas:** Derivatization. **III/Chiral Separations:** Chiral Derivatization; Cyclodextrins and Other Inclusion Complexation Approaches; Ion-Pair Chromatography; Ligand Exchange Chromatography; Liquid Chromatography; Supercritical Fluid Chromatography; Synthetic Multiple Interaction ('Pirkle') Stationary Phase.

Further Reading

Gil-Av E (1975) Present status of enantiomeric analysis by gas chromatography. *Journal of Molecular Evolution* 6: 131–144.

Helmchen G, Hoffmann RW, Mulzer J and Schaumann E (eds) (1995) *Houben-Weyl*, vol E 21a *Stereoselective Synthesis*, Schurig V *Gas Chromatography*, Stuttgart: Thieme, 168–187.

König WA (1987) *The Practice of Enantiomer Separation by Capillary Gas Chromatography*. Heidelberg: Hüthig.

König WA (1992) *Enantioselective Gas Chromatography with Modified Cyclodextrins*. Heidelberg: Hüthig.

König WA (1993) Enantioselective gas chromatography. *Trends in Analytical Chemistry* 12: 130–137.

Koppenhoefer B, Epperlein U and Schwierskott M (1997) *Fresenius Journal of Analytical Chemistry* 359: 107–114.

Schreier P, Bernreuther A and Huffer M (1995) *Analysis of Chiral Organic Molecules–Methodology and Applications*, Ch 3.5, pp. 132–233. Berlin: Walter de Gruyter.

Schurig V (1984) Gas chromatographic separation of enantiomers on optically active stationary phases. *Angewarde Chemie International Edition English* 23: 747–765.
Schurig V (1994) Enantiomer separation by gas chromatography on chiral stationary phases. *Journal of Chromatography* 666: 111–129.
Schurig V and Nowotny H-P (1990) Gas chromatographic separation of enantiomers on cyclodextrin derivatives. *Angewarde Chemie International Edition English* 29: 939–957.
Snopek J, Smolková-Keulemansová E, Cserháti T, Gahm K and Stalcup A (1996) Cyclodextrins in analytical separation methods. In: Atwood JL, Davies JED, MacNicol DD and Vögtle F (eds) *Comprehensive Supramolecular Chemistry*, vol. 3, ch. 18, pp. 515–571. Oxford: Pergamon.

Ion-Pair Chromatography

E. Heldin, Uppsala University, Uppsala, Sweden

Copyright © 2000 Academic Press

Introduction

In ion pair chromatography, the solute ion is distributed between the mobile and the stationary phase together with an ion of opposite charge (a so-called counterion). The technique is often used in reversed-phase chromatography as a convenient method to control the retention of solutes. The principle may, for example, be applied to direct chiral separations using either chiral or achiral counterions. When using an achiral counterion, the chromatographic system has to contain a chiral selector, e.g., a chiral stationary phase. The purpose of an achiral counterion then is to control the retention of the solute. However, the counterion may also influence the stereoselectivity by interactions with the chiral selector molecules. A chiral counterion may, on the other hand, be used with non-chiral stationary phases to promote chiral separation.

Ion Pairs: Principles

In order to distribute the solute molecules in a nonpolar environment, it has to be uncharged. However, if an ion of opposite charge is present in enough concentration the two ions may be distributed as a pair in the same way. An ion being double charged may be distributed together with two ions of single charge or one double charged, the only prerequisite being electroneutrality of the pair (**Figure 1**).

The equilibrium for a simple 1 : 1 ion pair may be given as:

$$HB^+ + C^- \leftrightarrow HBC$$

and is characterized by an extraction constant:

$$K_{ex} = \frac{[HBC]_{org}}{[HB^+]_{aq} \times [C^-]_{aq}} \qquad [1]$$

The nonpolar environment may be a liquid (mobile or stationary), a surface or a micelle in the liquid. For simplicity the principle for a liquid–liquid chromatographic system with aqueous mobile phase will be presented. Here the retention factor for the solute, k_{HB^+}, is equal to:

$$k_{HB^+} = D_{HB^+} \times (V_s/V_m) \qquad [2]$$

where D_{HB^+} is the distribution coefficient of the solute between the aqueous mobile phase and the organic stationary phase and V_s and V_m are the volumes of the two phases.

The distribution coefficient is defined as:

$$D = \frac{C_{org}}{C_{aq}} \qquad [3]$$

i.e., the ratio of the total concentration of solute in organic phase over the total concentration in aqueous phase. For the ion pair, D_{HB^+} may be expressed as:

$$D_{HB^+} = \frac{[HBC]_{org}}{[HB^+]_{aq}} = K_{ex} \times [C^-]_{aq} \qquad [4]$$

Thus, the retention factor will depend on the extraction constant of the ion pair and the concentration of counterion in the aqueous mobile phase. The magnitude of the extraction constants depends on the hydrophobicity of the solute and the counterion, the

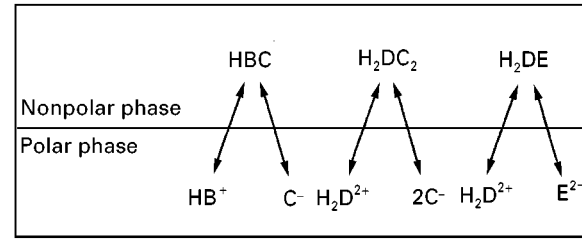


Figure 1 Distribution of charged compounds to a nonpolar phase together with a counterion.